

Genetics of Nervous System Disease

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ONE approach to understanding the function of presenilin 1 (PS1), is to discover those proteins with which it interacts. Evidence for a function in developmental patterning came from *C. elegans*, in which a PS homologue was identified by screening for suppressors of a mutation in *Notch/lin-12*, a gene which specifies cell fate. However, this genetic experiment cannot determine which proteins directly interact with PS1. Therefore, we utilized the two hybrid system and confirmatory co-immunoprecipitations to identify a novel catenin, termed δ -catenin, which interacts with PS1 and is principally expressed in brain. The catenins are a gene family related to the *Armadillo* gene in *Drosophila*, some of which appear to have dual roles—they are components of cell-cell adherens junctions, and may serve as intermediates in the Wingless (Wg) signaling pathway, which, like *Notch/lin-12*, is also responsible for a variety of inductive signaling events. In the non-neuronal 293 cell line, PS1 interacted with β -catenin, the family member with the greatest homology to *Armadillo*. Wg and *Notch* interactions are mediated by the *Dishevelled* gene, which may form a signaling complex with PS1 and Wg pathway intermediates to regulate the function of the *Notch/lin-12* gene.

Key words: Alzheimer's disease; *Armadillo*; Catenin; *Notch*; Presenilin; p120; Two hybrid; Wingless

Presenilin 1 interaction in the brain with a novel member of the Armadillo family

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Introduction

The gene most commonly mutated in early onset Alzheimer's disease, PS1, codes for presenilin 1, and harbors numerous different mutations.¹ Presenilin mutations increase the levels of the A β peptide which terminates at amino acid 42.²⁻⁴ Few clues regarding the normal function of PS1 are known. PS1 is a polytopic protein resident in the endoplasmic reticulum⁵ that generates two stable endoproteolytic fragments of ~27–28 kDa and ~17–18 kDa.⁶ Both its amino- and carboxytermini lie within the cytoplasm.^{7,8} PS1 is expressed in many tissues, but is enriched in the brain and in neurons by *in situ* hybridization, immunocytochemistry, and Northern blots.^{9,10} *C. elegans* contains a homologue of PS1 called *sel-12*, an allele of which was detected in a suppressor screen of *lin-12* gain-of-function mutations.¹ *lin-12* is the *C. elegans* homologue of the *Notch* gene, which provides critical patterning information during embryonic development in many different species. To enhance understanding of PS function we began with a two hybrid screen to identify proteins that directly interact with PS.

This search resulted in the discovery of the novel interacting gene, δ -catenin, which is related to the vertebrate gene β -catenin, a homologue of the *Drosophila* segment polarity gene *Armadillo*. These genes are characterized as a family by a series of imperfectly repeating 42 amino acid 'arm' motifs.¹² Some members of this family lie in the Wingless (Wg) pathway or in the homologous mammalian Wnt pathway, which also signal inductive events during development. Because intermediates in the Wg pathway can regulate *Notch*,¹³ the finding ties PS1 to a signaling cascade which may mediate the genetic observations in *C. elegans*.

Materials and Methods

Yeast two hybrid screening with the Matchmaker II kit (Clontech): Amino acids 263–407 of the PS1 'loop' were amplified by PCR with primers that added *EcoRI* and *BamHI* sites to the 5' and 3' ends and ligated into pAS2-1 to encode a hybrid protein containing the DNA-binding domain of GAL4 fused to PS1 residues 263–407. Yeast cells of the Y190 reporter strain were sequentially transformed with

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the PS1₂₆₃₋₄₀₇/pAS2-1 construct and then with an adult human brain expression library containing cDNAs fused to sequences of the GAL4 transactivation domain. Transformed Y190 cells were plated on SD/-His/-Trp/-Leu medium with 25 mM 3-AT (3-amino-1,2,4-triazole) and assayed for β -galactosidase activity.

In vitro interactions: cDNAs encoding A1, full length PS1 or the loop region of PS1 were ligated into the PSV-K3 expression vector for coupled *in vitro* transcription/translation in rabbit reticulocyte lysates using the TNT kit (Promega, Madison WI). Labeled polypeptides were mixed in binding buffer (10 mM Tris pH 8.0, 150 mM NaCl, 5 mM EDTA, 1 mM DTT, 1 mM PMSF, 10 μ g ml⁻¹ leupeptin, 10 μ g ml⁻¹ pepstatin, 10 μ g ml⁻¹ aprotinin), incubated with preimmune serum and protein A-Sepharose and then the supernatant was immunoprecipitated with polyclonal antibody 412 raised against a synthetic peptide spanning residues 336-345 of human PS1 (a generous gift from Dr Jesus Avila). Other experiments utilized a PS1 rabbit polyclonal antibody raised against GST fusion proteins of residues 1-80 and 263-407.

Glutathione-Sepharose beads bound to GST-fusion proteins were washed with binding buffer (10 mM Tris-HCl, pH 8.0; 200 mM NaCl; 5 mM EDTA, 0.5% NP-40, 1 mM DTT, 3 mg ml⁻¹ of BSA, and the above proteinase inhibitors), rocked with aliquots of *in vitro* translated [³⁵S]methionine-labeled proteins for 1 h at 4°C in binding buffer. The beads were washed with binding buffer, boiled in sample buffer, and analyzed by SDS-PAGE.

Northern blot: Multiple Tissue Northern Blot was purchased from Clontech. About 2 μ g poly A+ RNA was loaded to each lane. The A1 cDNA was labeled with ³²P and hybridized to the filter.

Results

Amino acids 263-407 of PS1 were selected as the bait for the two hybrid system because they were predicted by hydropathy analyses to lie in a loop that projects from the membrane and are therefore, more likely to represent an interactive domain. This sequence was fused to the DNA-binding domain of GAL4 to screen approximately 4 million human brain library transformants from which eight clones that co-activate the GAL4-responsive *HIS3* and *lacZ* reporter genes of Y190 were isolated. Two of the

isolates interacted specifically in a yeast two-hybrid mating assay that utilized two irrelevant proteins, murine p53 as a positive control and pLAM 5'-1 human lamin C as a negative control. A nucleotide sequence analysis of these two independent isolates revealed a novel gene with homology to *Armadillo* (Fig. 1). One of these two clones was designated A1. The expression pattern of A1 as revealed by Northern blot, indicated a strong 5.5 kb band confined almost exclusively to brain; the only other tissue with detectable signal was pancreas (Fig. 2).

To confirm the validity of the interaction, the two proteins were shown to co-immunoprecipitate *in vitro*. cDNAs encoding A1, full length PS1 or the loop region of PS1 were subcloned into the PSV-K3 expression vector, and these plasmids were used for coupled *in vitro* transcription/translation in rabbit reticulocyte lysates using the TNT kit (Promega, Madison WI). Translation of the plasmids in the presence of [³⁵S] methionine generated a radiolabeled A1 polypeptide of ~45 kDa, a PS1 band of ~43 kDa, and a PS1 loop peptide of 16 kDa. Various combinations of these *in vitro* translated proteins were mixed in binding buffer (see Materials and Methods) and immunoprecipitated. The antibody immunoprecipitated the PS1 loop peptide with A1 (Fig. 3) and full length PS1 with A1. An *in vitro* interaction was also demonstrated by mixing equivalent aliquots of the radiolabeled protein with purified glutathione S-transferase (GST), or with purified GST-fusion proteins containing either A1, an amino terminal PS1 construct from amino acid 1-80, or residues 263-407 of PS1. Each mixture of GST proteins were adsorbed to glutathione-agarose beads. *In vitro* translated A1 was specifically retained on the beads by the PS1 loop-GST fusion protein, but not by the parental GST polypeptide or by GST fused to the amino terminus of PS1 (Fig. 3). The reverse experiment demonstrated that *in vitro* translated PS1 residues 263-407 specifically bound to the A1-GST fusion protein (Fig. 3).

To demonstrate an interaction *in vivo* with an endogenous member of the Armadillo family, we utilized 293 cells for co-immunoprecipitation experiments. Although these cells have undetectable levels of A1 (data not shown), they have relatively high levels of other catenins. Two different PS1 antibodies precipitated β -catenin (Fig. 4), but failed to co-immunoprecipitate α -catenin or γ -catenin (data not shown). In the reverse experiment an antibody to β -catenin co-immunoprecipitated PS1 (Fig. 4). We

FIG. 1. The assembled sequence of γ -catenin. Brackets mark the arm repeats. The shaded region denotes the coding region of the original A1 clone identified in the two hybrid screen and the shaded arrow marks the end of the A1 clone. The sequence AATAAA is a putative poly A signal 29 base pairs from the end of the sequence. The boxed tyrosine (Y) lies within a phosphorylation consensus sequence.

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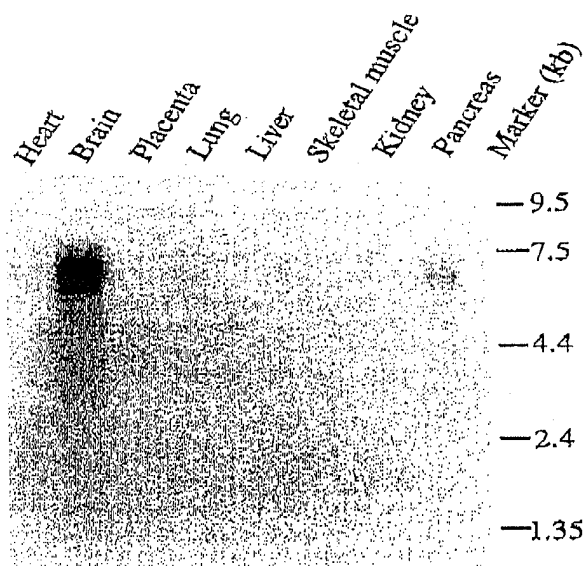
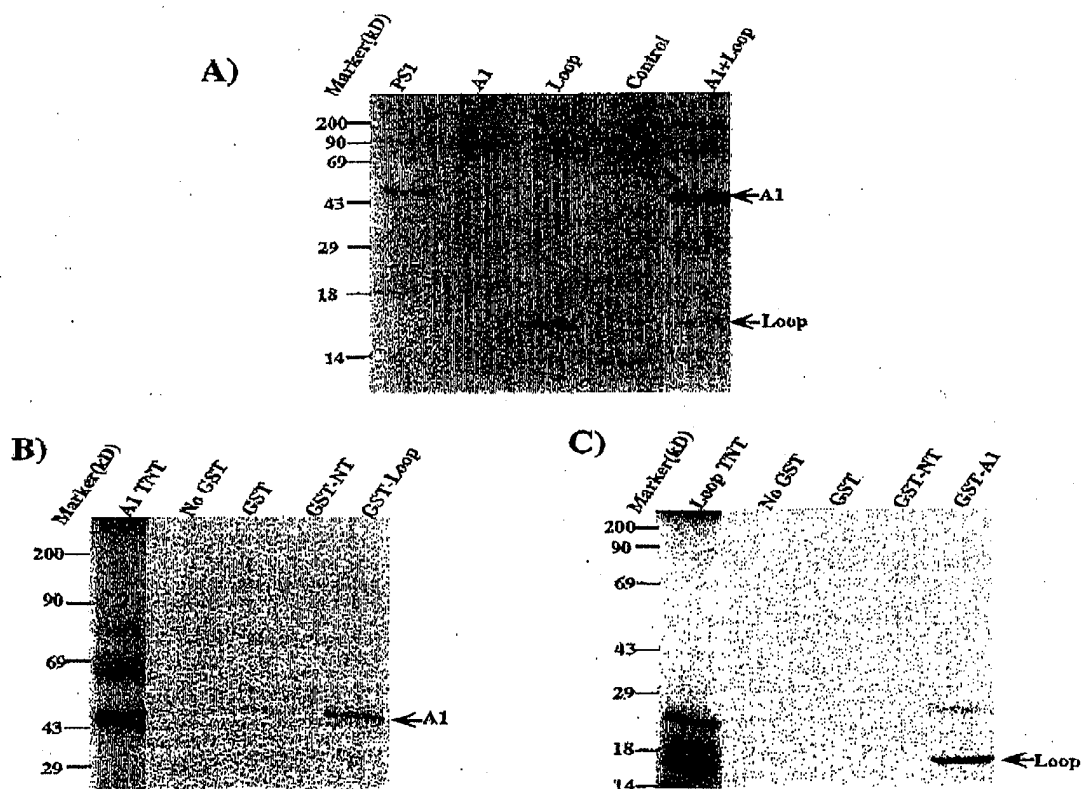


FIG. 2. Northern blot analysis of δ -catenin expression in different human tissues. The δ -catenin is expressed mostly in brain and much less in pancreas. The major band is about 5.5 kb; minor bands are present at 4.5 kb and 7 kb.

FIG. 3. *In vitro* co-immunoprecipitation. (A) Each lane shows samples immunoprecipitated by PS1 antibody 412: *in vitro* translated PS1 is precipitated, *in vitro* translated A1 alone is not precipitated, residues 263-407 (loop) is precipitated, *in vitro* translated luciferase protein (control) is not precipitated, a mixture of A1 and loop (arrows) are co-immunoprecipitated by antibody 412. (B and C) Binding to glutathione-S-transferase (GST) fusion proteins: A1 TNT is the *in vitro* translated product of clone A1; Loop TNT is the *in vitro* translated product of PS1 residues 263-407; lanes labeled no GST, GST, and GST-NT demonstrate the lack of A1 binding (B) or loop binding (C) to glutathione beads mixed with host bacterial extract (no GST), GST alone (GST), and GST fused to the amino terminus of PS1 (GST-NT); the lane labeled GST-loop demonstrates the binding of A1 to residues of 263-407 of PS1 (B); the lane labeled GST-A1 demonstrates the binding of PS1 loop to A1.



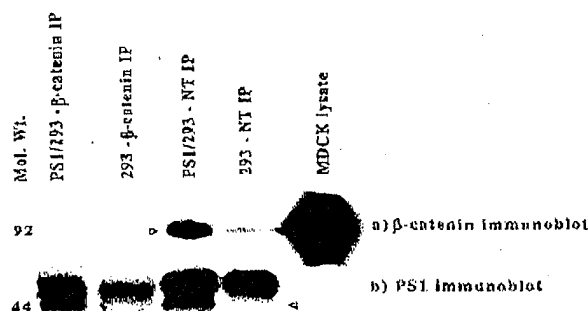


FIG. 4. Co-immunoprecipitation of endogenous β -catenin with PS1 in 293 cells. (a) The open arrowhead indicates immunostained β -catenin immunoprecipitated (IP) with an amino terminal (NT) PS1 antibody. Greater amounts of β -catenin are present in 293 cells transfected with PS1 (PS 1/293) compared to non-transfected cells. In MDCK cell lysates there is a large amount of β -catenin. (b) Arrowhead indicates co-immunoprecipitation of PS1 with β -catenin antibody in PS1 transfected cells (PS 1/293) and with the PS1 NT antibody. Note the larger band above PS1 is present in all lanes and represents the IgG heavy chain.

concluded that PS1 can form stable complexes *in vivo* with specific members of the Armadillo family and the particular binding partner may vary depending upon the cell type.

Having demonstrated the authenticity of the interaction we sought to obtain a full length sequence for A1. Portions of the 3' end of the A1 sequence were present in the database in a clone designated NIBA2. This clone was obtained from two YACs that spanned the ~2 Mb cri-du-chat critical region (CDCCR) of chromosome 5p¹⁴ and were used to screen high density arrays of clones directly from an arrayed normalized infant brain (NIB) cDNA library.¹⁵ This led to the identification of a ~2.0 kb cDNA, designated NIBA2, that mapped back to YAC 938G6 but beyond the boundaries of the CDCCR.¹⁶ To extend A1 in the 5' direction we utilized human adult brain Marathon cDNA with Advantage Taq polymerase (Clontech, CA) and 5-Race PCR (Gibco) and assembled the entire sequence (Fig. 1). This sequence, termed δ -catenin, shows greatest homology to p0071¹⁷ (69.3% identical, EMBL GenBank accession number X81889) and somewhat less homology to p120¹⁸ (48.0% identical, accession number Z17804) two proteins along with B6P/plakophilin 1 (accession number X79293), that are considered a subfamily of the catenins. This very close homology to p0071 is even greater than the 46.4% identity between p0071 and p120. Each of these proteins has 10 repeat arm domains that span 482 amino acids for δ -catenin, 479 amino acids for p0071, and 554 amino acids for p120. The arm

domains of these proteins are believed to serve as distinct signaling motifs.¹⁹ Interactions of PS1 with β -catenin or δ -catenin may be a function of the cell type or may arise from a common binding site in both proteins. Notable in this regard, arm domain four of β -catenin and δ -catenin share 66% similarity.

Direct interaction of PS1 with members of the Armadillo family has a number of implications. The mammalian gene coding for β -catenin, which bears the greatest homology to the *Drosophila* gene *Armadillo*, is present in at least two intracellular pools. One pool is located in the adherens junction where β -catenin is bound to cadherin; a second pool lies within the cytoplasm as a downstream effector of the Wg/Wnt signaling pathway. Within the cell, both presenilin⁵ and members of the Wnt family²⁰ are retained in the endoplasmic reticulum. Both of these pools represent potential sites to search for interactions that may lead to Alzheimer's disease. The relatively specific brain expression of δ -catenin, in contrast to the more generalized expression of PS1, PS2, and APP, may explain the targeting of the disease to the brain. One site unique to nervous tissue where catenins are found is a subdomain of the synaptic junction bordering the transmitter release zone in a structure referred to as the synaptic adherens junction.²¹ Synapses represent a target site of Alzheimer disease pathology²²⁻²⁴ and plasma membranous APP is localized to sites of cell-cell contact.²⁵ This confluence of data pointing to the synaptic adherens junction suggests this site as a potential locus of disease initiation.

The presenilins also have a role in *Notch* signaling because an allele of the *C. elegans* gene *sel-12*, a presenilin homologue, can suppress *Notch/lin-12* gain-of-function mutations.¹¹ The effect of *sel-12* on the *Notch* pathway may operate via direct interactions in the Wg pathway that regulates the implementation of the *Notch* signal. One mediator of signals between these pathways is *Disheveled*, which transduces the Wingless signal and blocks the *Notch* signal.¹³ PS1 represents a second potential link between these two key developmental pathways.

Conclusion

PS1 interacts with a novel member of the Armadillo family termed δ -catenin, a protein expressed mostly in brain and encoded on chromosome 5. The gene was identified on a two hybrid screen and confirmed to exist as a complex by additional binding studies. The interaction involves the 'loop' region of PS1. In the non-neural 293 cell line, PS1 and another member of the Armadillo family, β -catenin, co-immunoprecipitate. Genes of the Armadillo

family lie downstream in the Wingless pathway and provide patterning information in early development. Interactions between PS1 and members of the Armadillo gene family may form signaling complexes that contribute to the known effects of the presenilin homologue, *sel-12*, on the *Notch* pathway.¹

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General Summary

Among the causes of early onset Alzheimer's disease (AD) are mutations in three different genes: the amyloid precursor protein gene, presenilin 1 (PS1) and presenilin 2 (PS2). Mutations in PS1 are the most common known cause of early onset AD; however, little is known about the normal function of this gene. One means to learn about a protein's function is to discover the company it keeps. We found a PS1 binding partner that is in a gene family involved in the early development of the body plan. The family member identified here is expressed mostly in the brain and may ultimately contribute to understanding why AD selectively attacks the brain.